

Fig. 1. is a table showing the emission wavelength maximum (nm) of aequorin mutants with coelenterazine analogues.

Coelenterazine Analogue	Wild Type Aequorin	Aequorin Mutant S (Aeq3)	Aequorin Mutant S Ser5Cys (Aeq5)	Aequorin Mutant S Tyr132Ile
CTZ i	472	491	491	487
CTZ ip	472	470	454	453
CTZ h	472	476	471	471
CTZ hcp	472	476	448	465
CTZ cp	472	470	456	457
CTZ fcp	472	466	471	471
CTZ f	472	490	473	471
CTZ n	472	487		
CTZ native	472	474	471	471

* All values, except wild type aequorin, were calculated from the average of 3 trials (wild type with 2). All mutants were, except Aeq5 purified to >95% purity. CTZ analogues diluted to 100 micrograms/ml methanol.

FIG. 2
Emission Spectra of Aeq3 and Native Aeq With CTZ i, hcp

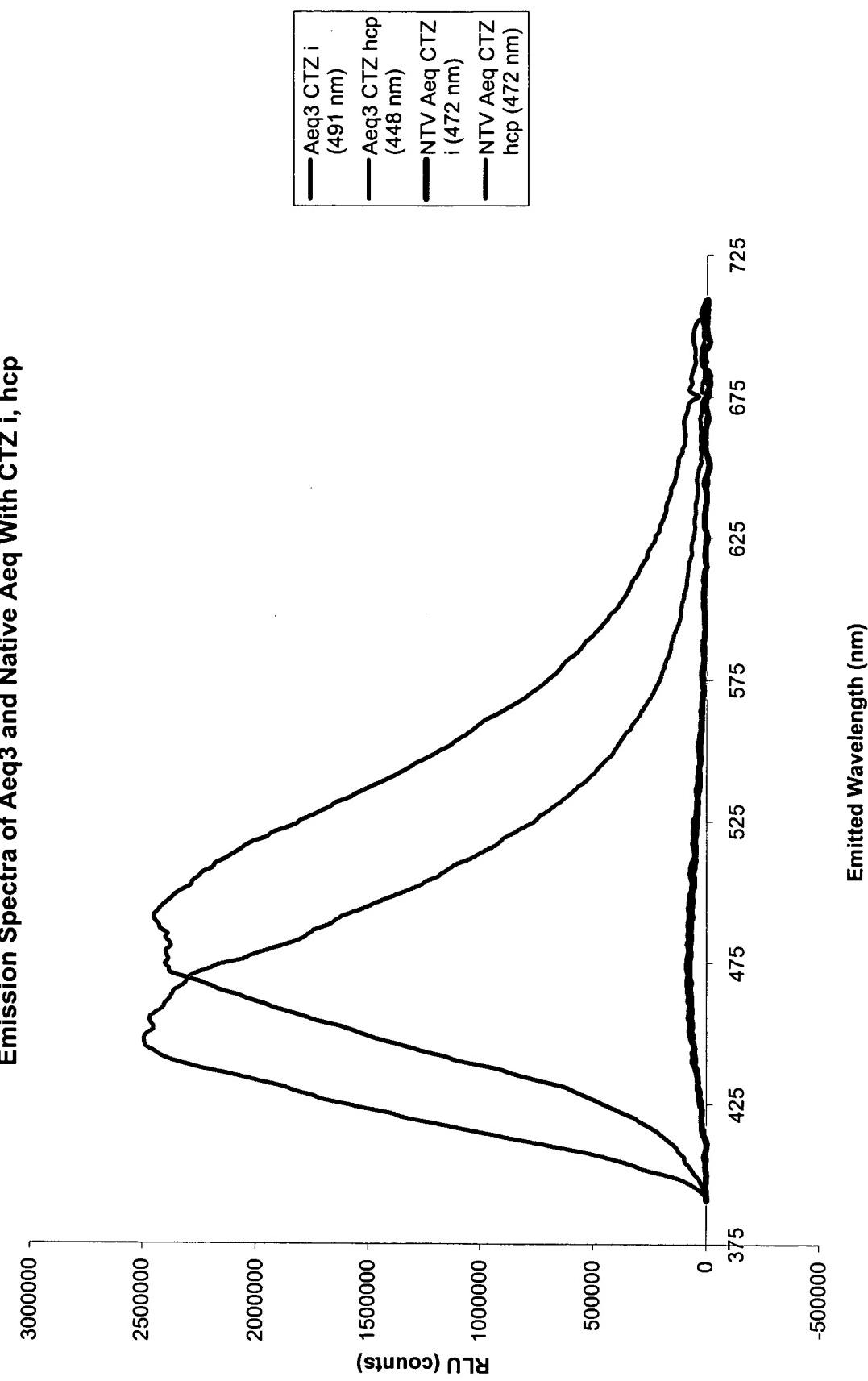
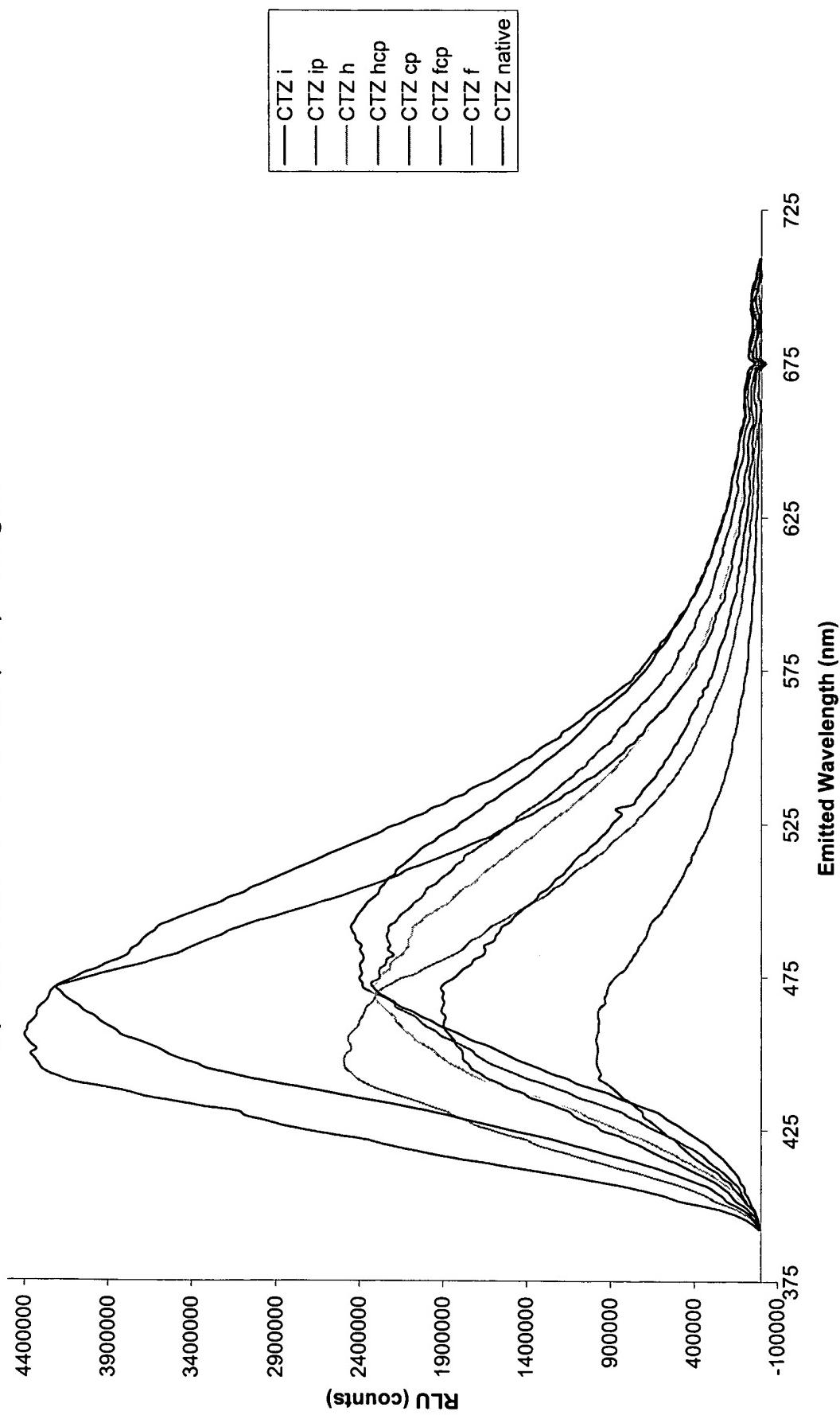


FIG. 3
Aequorin 3 With Coelenterazine (CTZ) Analogues



Crude Aequorin 5 (Average of 2 Trials) With Coelenterazine (CTZ) Analogues

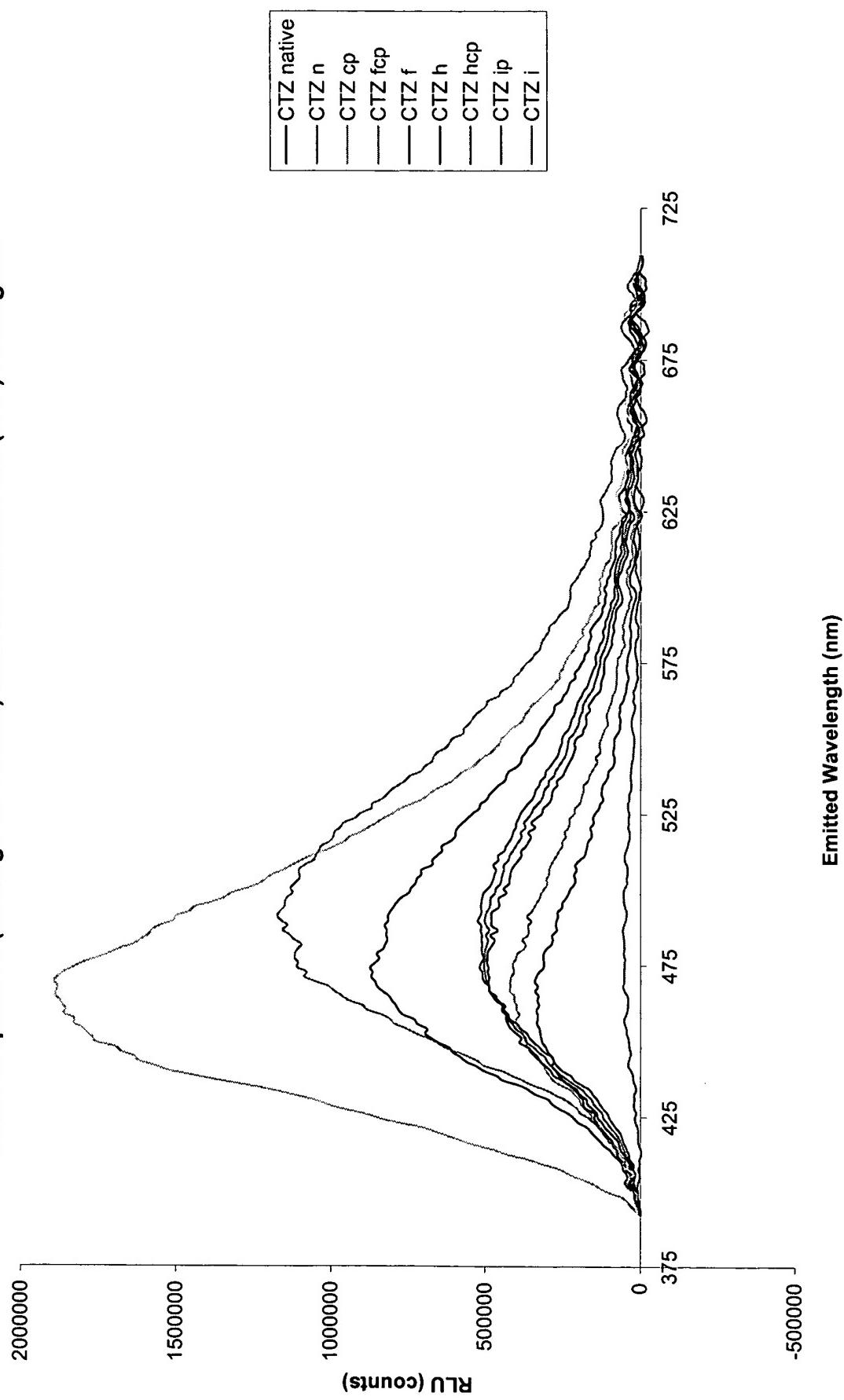


FIG. 5
Aequorin Y132I Aequorin With Coelenterazine (CTZ) Analogue

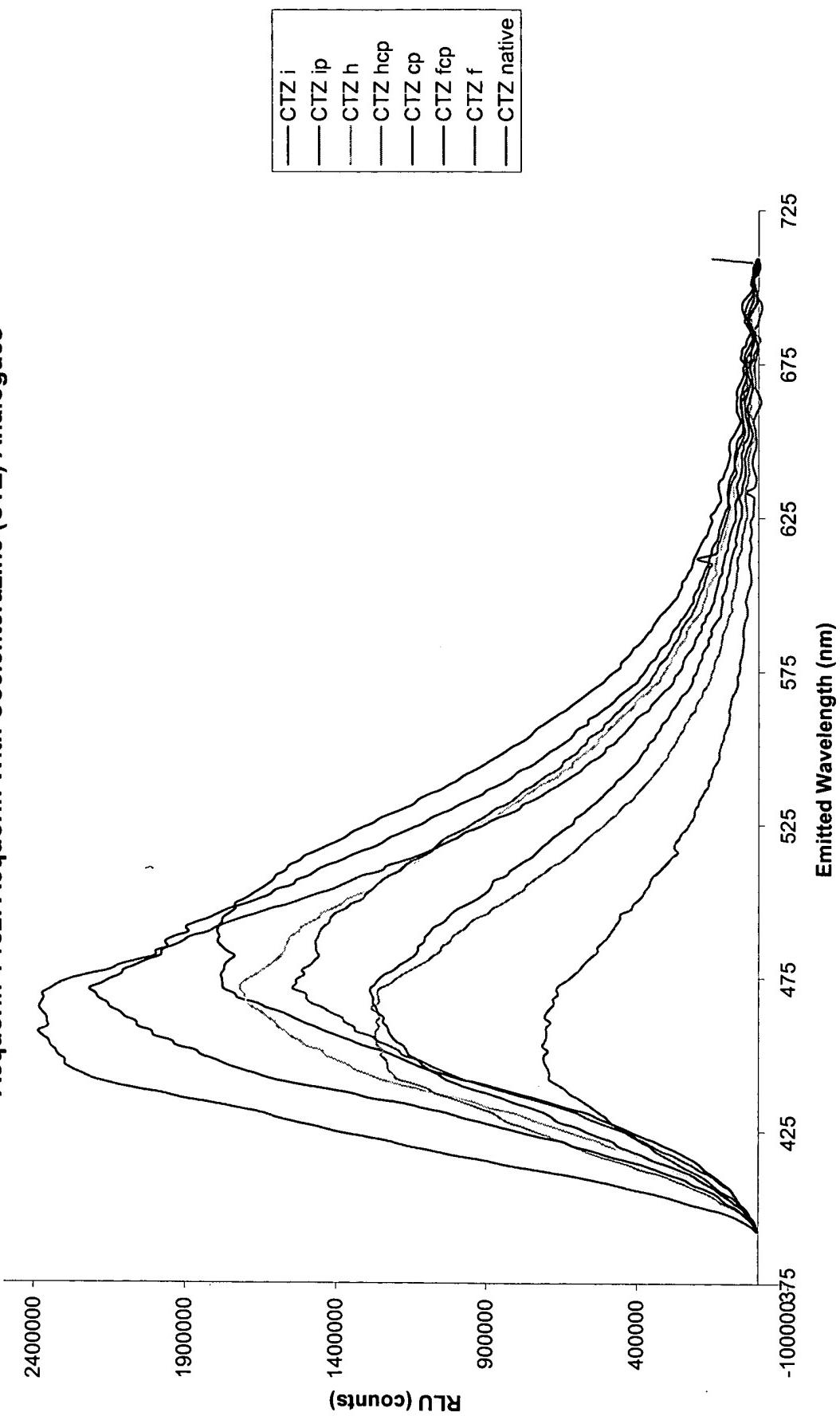


FIG. 6
Native Aequorin with Coelenterazine (CTZ) Analogues

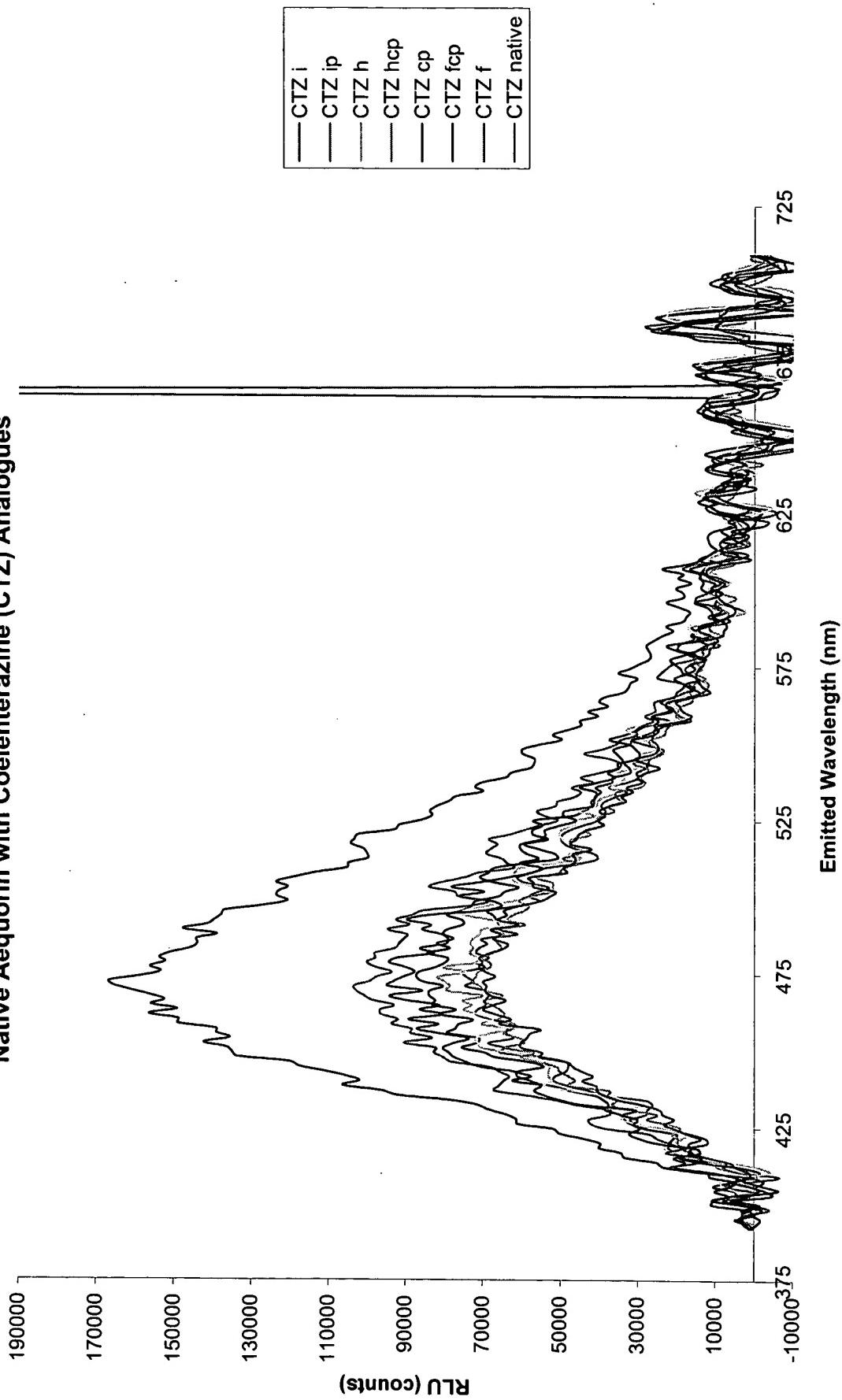


Fig. 7. is a table showing the emission wavelength maximum (nm) of aequorin mutant Mutant S Y132I, Mutant S having a 3-fluoro-l-tyrosine aequorin or a 5-fluoro-l-tyrosine non-natural amino acid in position 132 in conjunction with coelenterazine analogues CTZ i, ip, n, h, hcp, cp, fcp, f and native CTZ.

Coelenterazine Analogue	Wild Type Aequorin	Aequorin Mutant S Tyr132Ile	Aequorin Mutant S Tyr132 3-fluoro-l-tyr	Aequorin Mutant S Tyr132 5-fluoro-l-trp
CTZ i	472	491	511	495
CTZ ip	472	452	471	
CTZ n	472	491	500	
CTZ h	472	472	498	471
CTZ hcp	472	452	471	468
CTZ cp	472	457	471	471
CTZ fcp	472	463	471	
CTZ f	472	472	500	497
CTZ native	472	471	495	472

Figure 8
Spectra of 3-Fluoro-L-Tyrosine

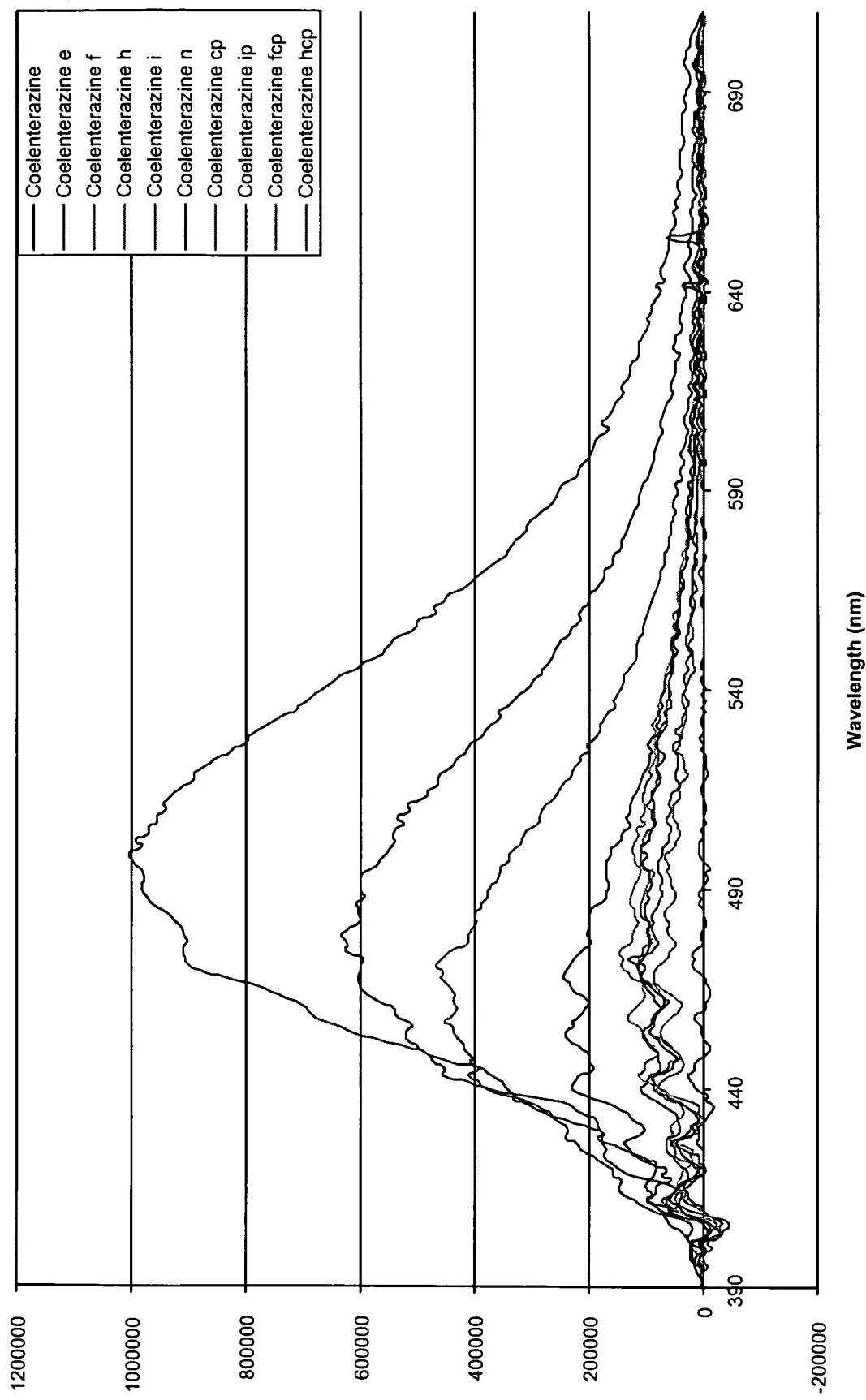


Figure 9
Spectra of 5-Fluoro-L-Tryptophan

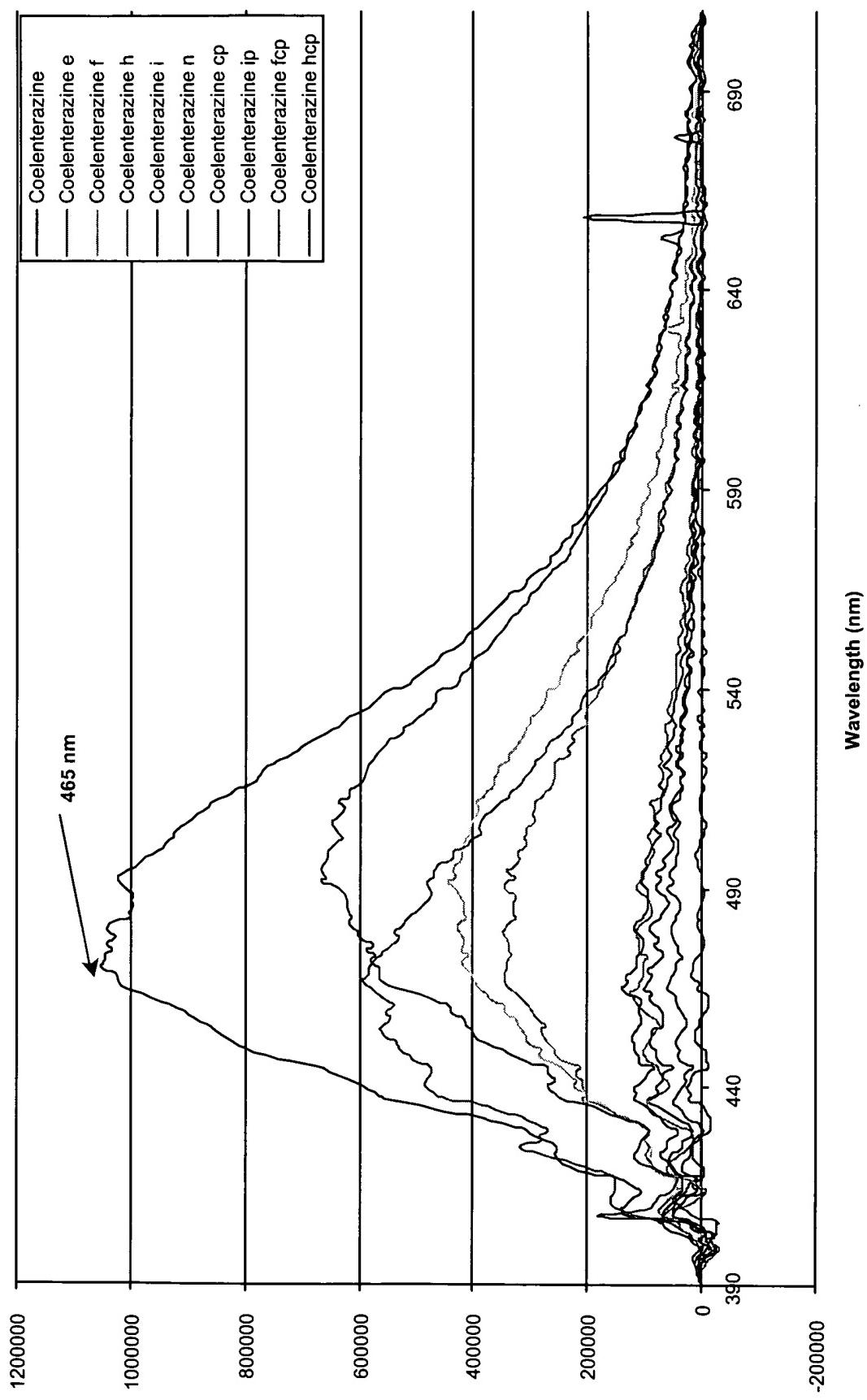


Fig. 10

Energy Transfer Study: Aequorin Mutants

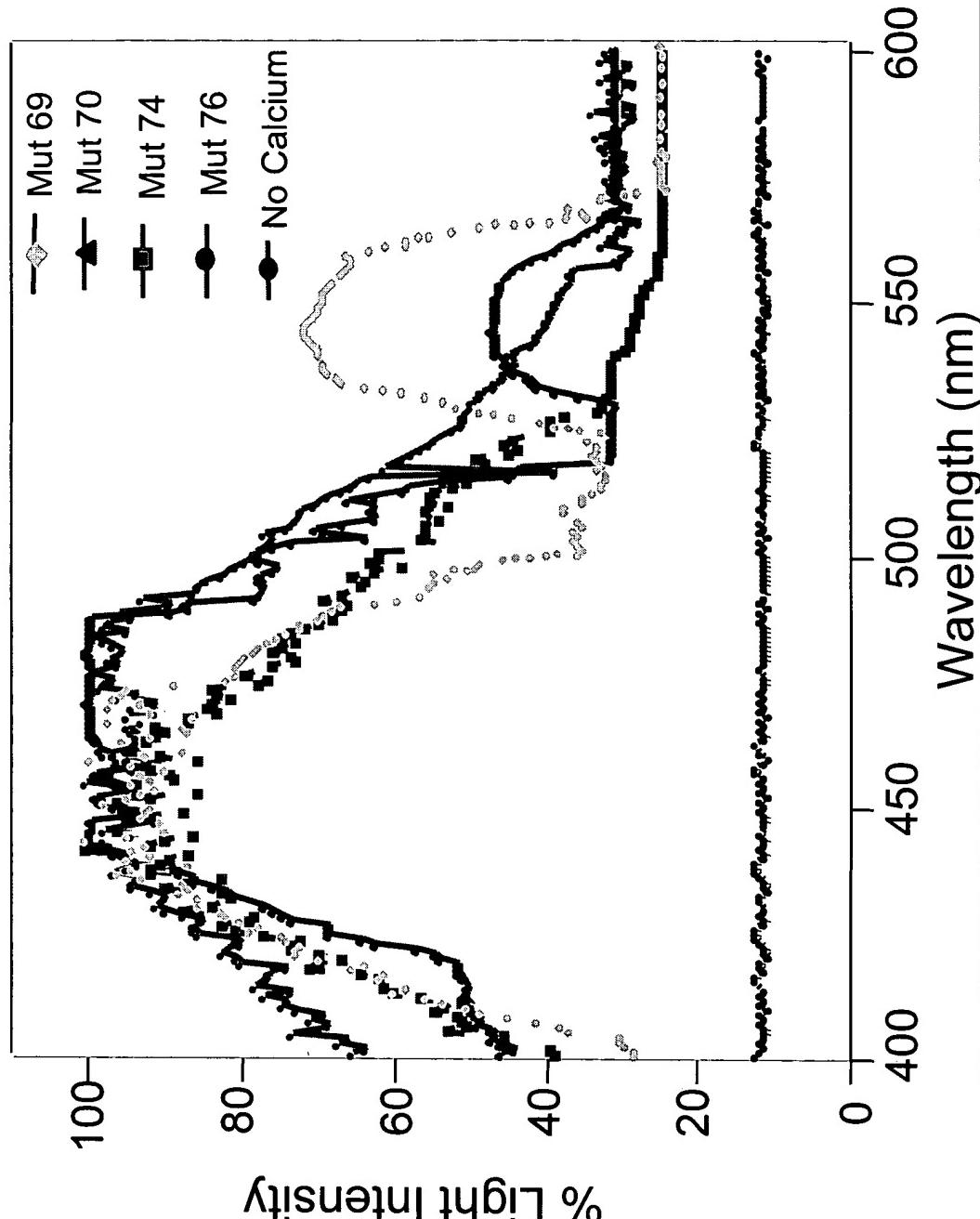


Fig. 11: SEQ ID NO: 1 cDNA encoding wild type apoaequorin

1 aatgcaattc atctttgcat caaagaatta catcaaatct
ctagttgatc aactaaattg
61 ttcgcacaac aacaagcaaa **catg**acaaggc aaacaatact
cagtcaagct tacatcagac
121 ttcgcacaacc caagatggat tggacgacac aagcatatgt
tcaatttcct tgatgtcaac
181 cacaatggaa aaatctctct tgacgagatg gtctacaagg
catctgatat tgtcatcaat
241 aaccttgag caacacctga gcaagccaaa cgacacaaag
atgctgtaga agccttcttc
301 ggaggagctg gaatgaaata tgggtggaa actgattggc
ctgcatatat tgaaggatgg
361 aaaaaatgg ctactgatga attggagaaa tacGCCaaa
acgaaccaac gctcatccgt
421 atatgggtg atgctttgtt tgatatcggt gacaaagatc
aaaatggagc cattacactg
481 gatgaatgga aagcatacac caaagctgct ggtatcatcc
aatcatcaga agattgcgag
541 gaaacattca gagtggtgcga tattgatgaa agtggacaac
tcgatgttga tgagatgaca
601 agacaacatt taggattttg gtacaccatg gatcctgctt
gcgaaaagct ctacggtgaa
661 gctgtccc**t** aagaagctct acgggtggta tgcaccctgg
gaagatgatg tgattttgaa
721 taaaacactg atgaattcaa tcaaaatttt ccaaattttt
gaacgatttc aatcgttgt
781 gttgattttt gtaatttagga acagattaaa tcgaatgatt
agttgtttt ttaatcaaca
841 gaacttacaa atcgaaaaag t

Fig. 12: SEQ ID NO: 2 amino acid sequence for wild type apoaequorin

VKLTSDFDNP RWIGRHKHMF NFLDVNHNGK ISLDEMVKYKA SDIVINNLGA
TPEQAKRHKD AVEAFFGGAG MKYGVETDWP AYIEGWKKLA TDELEKYAKN
EPTLIRIWGD ALFDIVDKDQ NGAITLDEWK AYTKAAGIIQ SSEDCEETFR
VCDIDESGQL DVDEMTRQHL GFWYTMMPAC EKLYGGAVP

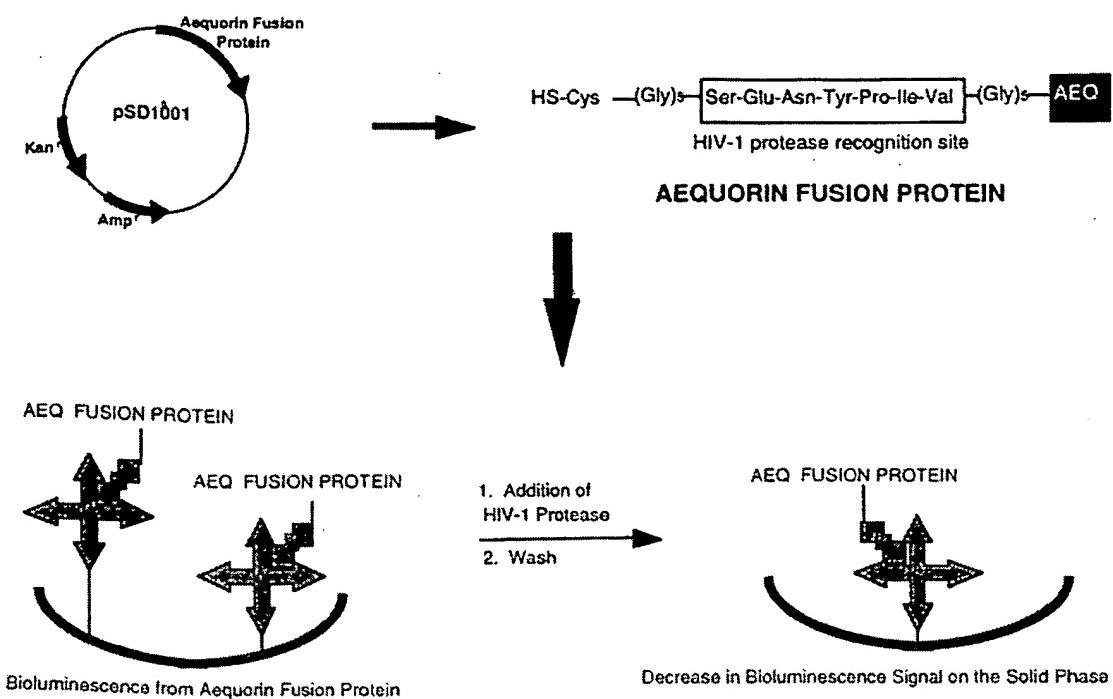
Fig. 13: SEQ ID NO: 3 cDNA encoding Mutant S apoaequorin

```
1 aatgcaattc atcttgcat caaagaatta catcaaatct  
ctagtgtatc aactaaattt  
61 tctcgacaac aacaagcaaa catgacaaggc aaacaatact  
cagtcaagct tacatcagac  
121 ttgcacaacc caagatggat tggacgacac aagcatatgt  
tcaatttcct ttagtgtcaac  
181 cacaatggaa aaatctctct tgacgagatg gtctacaagg  
catctgatat tgtcatcaat  
241 aaccttggag caacacctga gcaagccaaa cgacacaaag  
atgctgtaga agccttcttc  
301 ggaggagctg gaatgaaata tgggtggaa actgattggc  
ctgcatatat tgaaggatgg  
361 aaaaaattgg ctactgatga attggagaaa tacgccaaaa  
acgaaccaac gctcatccgt  
421 atatgggtg atgctttgtt tgatatcggt gacaaagatc  
aaaatggagc cattacactg  
481 gatgaatgga aagcatacac caaagctgct ggtatcatcc  
aatcatcaga agatagcgag  
541 gaaacatcca gagtgagcga tattgatgaa agtggacaac  
tcgatgttga tgagatgaca  
601 agacaacatt taggatttg gtacaccatg gatcctgcta  
gcaaaaagct ctacggtgaa  
661 gctgtccct aagaagctct acgggtggta tgccacctgg  
gaagatgatg tgatttgaa  
721 taaaacactg atgaattcaa tcaaaatttt ccaaattttt  
gaacgatttc aatcgttgt  
781 gttgattttt gtaatttagga acagattaaa tcgaatgatt  
agttgtttt ttaatcaaca  
841 gaacttacaa atcgaaaaag t
```

Fig. 14: SEQ ID NO: 4 amino acid sequence for “Mutant S” apoaequorin

VKLTSDFDNP RWIGRHKHMF NFLDVNHNGK ISLDEM VYKA SDIVINNLGA
TPEQAKRHKD AVEAFFGGAG MKYGVETDWP AYIEGWKKLA TDELEKYAKN
EPTLIRIWGD ALFDIVDKDQ NGAITLDEWK AYTKAAGIIQ SSED**SEETFR**
VSDIDESGQL DVDEMTRQHL GFWYTM DPAS EKLYGGAVP

Fig. 15



Plasmid construct for the expression of the aequorin fusion protein and schematic representation of the fusion protein showing the HIV-1 protease cleavage site. B represents biotin and NA represents Neutravidin immobilized on the wells.

Fig. 16: Coelenterazine Analogs

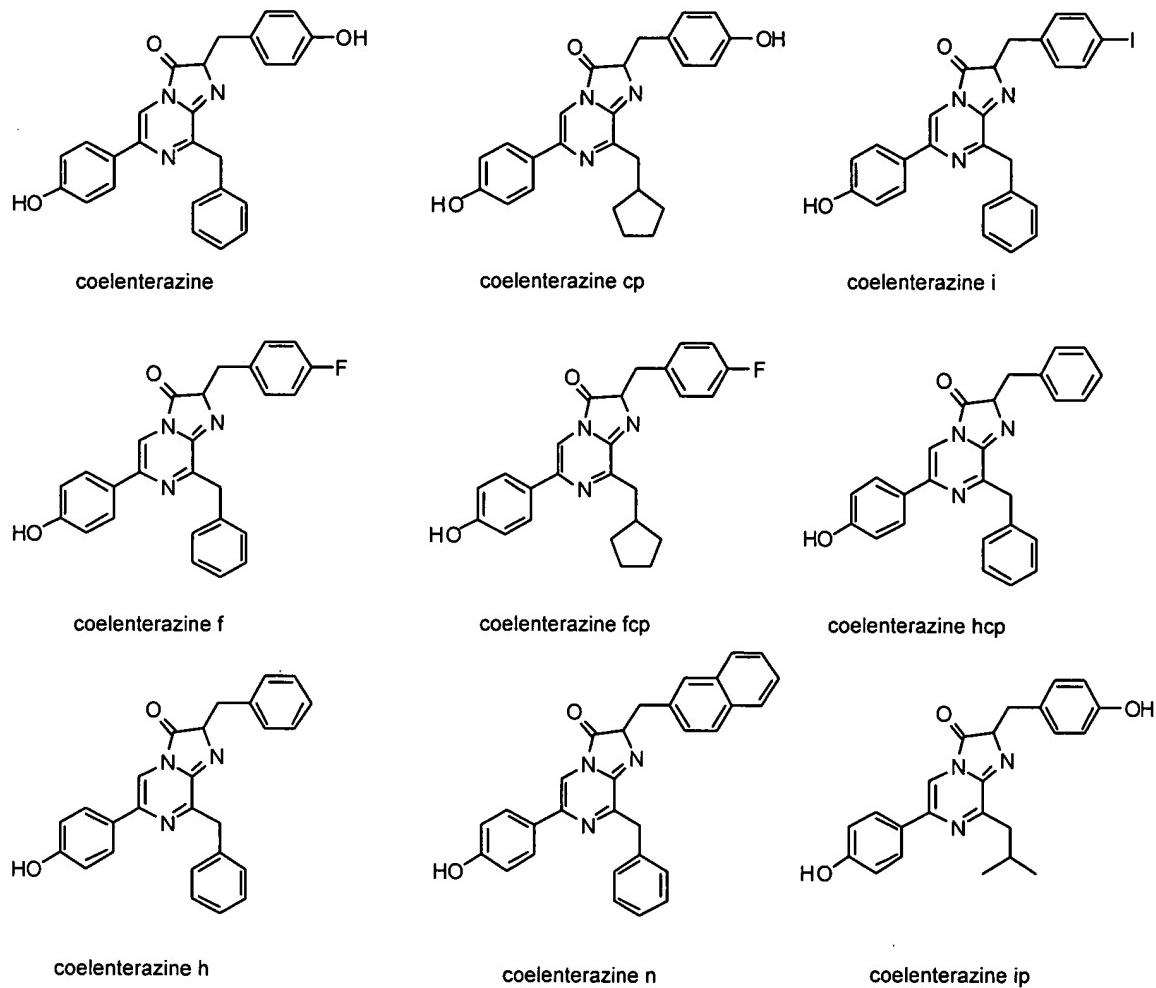


Fig. 17. Non-natural amino acids

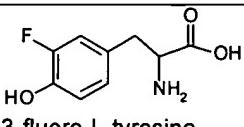
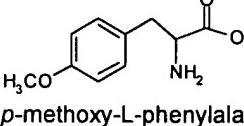
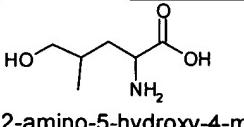
Non-natural Amino Acid Analogue	Representative Example	Effect
Fluoro-analogue	 3-fluoro-L-tyrosine	Altered pK _a and H-bonding strength
Amine-analogue	 p-amino-L-phenylalanine	Altered electrostatic charge and H-bonding strength
Alkyl-analogue	 p-methoxy-L-phenylalanine	Altered H-bonding capability, increased bulkiness
Nitro-analogue	 2-nitro-L-tyrosine	Altered π-character, altered pK _a , altered H-bonding strength, and steric effects
Hydroxyl-analogue	 2-amino-5-hydroxy-4-methyl pentanoic acid	Altered pK _a , loss of π-π interactions

Fig. 18 SEQ ID NO: 5, Obelin cDNA sequence

1 acgatcgaac caaacaactc agctcacagc tactgaacaa
ctcttgttgt gtacaatcaa
61 aatgtcttca aaatacgcag tttaaactcaa gactgacttt
gataatccac gatggatcaa
121 aagacacaag cacatgtttg atttcctcg a catcaatgga
aatggaaaaa tcaccctcg a
181 tgaaattgtg tccaaggcat ctgatgacat atgtgccaa g
ctcgaagcca caccagaaca
241 aacaaaacgc catcaagttt gtgttgaagc tttctttaga
ggatgtggaa tggaaatatgg
301 taaagaaatt gccttcccac aattcctcg a tggatggaaa
caattggcga cttcagaact
361 caagaaatgg gcaagaaacg aacctactct cattcgtgaa
tggggagatg ctgtcttga
421 tatttcgac aaagatggaa gtggtacaat cactttggac
gaatggaaag cttatggaaa
481 aatctctggt atctctccat cacaagaaga ttgtgaagcg
acatttcgac attgcgattt
541 ggacaacagt ggtgaccttg atgttgcga gatgacaaga
caacatcttg gattctggta
601 cactttggac ccagaagctg atggtctcta tggcaacgga
gttccctaag cttttttcg
661 aa

Fig. 19. SEQ ID NO: 6, Native Obelin Amino Acid Sequence

MSSKYAVKLK TDFDNPRWI**K** RHKHMFD**L**D INGNGKITLD EIVSKASDDI
CAKLEATPEQ TKRHQVCVEA FFRGC**G**MEYG KEIAFPQFLD GWKQLATSEL
KKWARNEPTL IREWGD**A**VFD IFDKDGSGTI TLDEWKAYGK ISGISPSQED
CEATFRH**CD**L DNSGDLDVDE MTRQHLGF**W**Y TLDPEADGLY GNGVP

Fig. 20 Obelin mutants with Coelenterazine analogues.

Obelin Mutant	Coelenterazine Analogs Emission Max (nm)							
	<i>i</i>	<i>ip</i>	<i>h</i>	<i>hcp</i>	<i>cp</i>	<i>fcp</i>	<i>f</i>	<i>ctz</i>
C75S- C51S	521	478	503	478	472	497	503	491
C75S- C67S	505	471	500	471	471	487	504	491
C158S	506	472	497	478	472	475	497	491
C151S	497	471	490	471	471	474	493	491